INFORMATION AND INSTRUCTIONS

1. Regarding the objection to the claim pendency, please amend the claims in accordance with your practice.

2. Regarding the prior art rejection, please amend the main claim (claim 31) as follows to overcome the cited reference.

"31. A therapeutic method for treatment of acute lung injury resulting from indirect causes which occur systemically and thereby injure the lung indirectly, which method comprises administering anti-IL-8 antibody to a subject in need of said therapy."

In the amended claim 31, the "indirect causes" are further defined as --indirect causes which occur systemically and thereby injure the lung indirectly--, as supported by the description on page 31, lines 33 to 34 of the specification, as well as page 32, line 24, Example 1 (pages 36 to 37). If necessary, please amend the other claims to make them conform to the amended claim 31.

The cited reference, Folkessor H.G., et al., refers to direct injury of the lung caused by acid aspiration. On the other hand, the present invention is directed to treatment of indirect injury of the lung.

It is well recognized and accepted in the art that the indirect injury of the lung and the direct injury thereof are completely different. For example, according to the Conference Report of The American-European Consensus Conference on ARDS (Bernard G.R., et al., Am. J. Respir. Crit. Care Med. 149, p. 818 - 824 (1994)) (see, page 3, lines 15 to 20 of the English specification), ARDS and ALI are categorized on the basis of their causes to "direct

injury" and "indirect injury" (see, page 821, the left column the bottom portion to the right column the top portion).

Please note that an effect or action of a drug to the direct injury and an effect or action of the same drug to the indirect injury are completely different. For example, Nishina K. et al., Anesthesiology, Vol. 88, p. 1300 - 1309, 1998, ABSTRACT (enclosed), describe that administration of Lidocaine to a direct injury caused by acid aspiration is effective. On the other hand, K. Nishina et al., Anesthesiology, Vol. 83, p. 169 - 177 (1995) (enclosed) describe that administration of Lidocaine to indirect injury caused by endotoxin infusion is not significantly effective.

Accordingly, even though the cited Folkessor et al. reference shows that anti-IL-8 antibody is effective to direct injury, this fact does not suggest that the anti-IL-8 antibody is effective to indirect injury. Therefore, the present invention of the amended claims should be patentable over the citation.

Anesthesiology
1998; 88:1300-9

Intravenous Lidocaine Attenuates Acute Lung Injury Induced by Hydrochloric Acid Aspiration in Rabbits

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Background: Neutrophils play a crucial role in the pathogenesis of acid-induced acute lung injury. Lidocaine inhibits the function of neutrophils. This study aimed to determine whether lidocaine attenuates acute lung injury induced by hydrochloric acid (HCl) instillation.

Methods: In study 1, rabbits were divided into four groups (n = 7 each). Lung injury was induced by intratracheal HCl (0.1 N, 3 ml/kg) in two groups. The other two groups received saline intratracheally. Lidocaine given intravenously (2 mg/kg bolus + 2 mg · kg^{-1} · h^{-1} infusion) was started 10 min before intratracheal instillation in one HCl and one saline group, and saline was given intravenously in the other two groups. In study 2, rabbits (four groups of seven animals each) received HCl (0.1 N, 3 ml/kg) intratracheally. Treatment with intravenous lidocaine was started 10 min before, 10 min after, or 30 min after acid instillation, or saline was given intravenously 10 min before instillation.

Results: In study 1, HCl caused deterioration of the partial pressure of oxygen (Pa_{O_2}), lung leukosequestration, decreased lung compliance, and increased the lung wet-to-dry weight ratio and albumin, interleukin-6 (IL-6), and IL-8 levels in bronchoalveolar lavage fluid. Lidocaine pretreatment attenuated these changes. Hydrochloric acid increased superoxide anion production by neutrophils and caused morphologic lung damage, both of which were lessened by lidocaine. In study 2, lidocaine given 10 min after acid instillation was as effective as pretreatment in Pa_{O_2}, lung mechanics, and histologic examination. However, Pa_{O_2} changes in lidocaine 30 min after injury were similar to those in saline given intravenously.

Conclusions: Intravenous lidocaine started before and immediately after acid instillation attenuated the acute lung injury, in part by inhibiting the sequestration and activation of neutrophils. (Key words: acid aspiration; acute respiratory distress syndrome; edema; immune response; local anesthetics; neutrophils; superoxide anion.)

ACID aspiration may produce severe, acute lung injury, the characteristics of which resemble those of acute respiratory distress syndrome.[1] The precise mechanism through which acid aspiration induces respiratory failure is not yet fully understood. However, cellular components (*e.g.*, neutrophils, macrophages) and humoral mediators (*e.g.*, cytokines) are thought to play a pivotal role in the pathogenesis of this acute lung injury.[2-5] Neutrophils accumulated in the lung in response to chemotaxins adhere to pulmonary endothelial cells through the interaction of adhesion molecules, the expression of which is enhanced by cytokines.[6,7] Neutrophils thereafter attack the endothelium by releasing protease, reactive oxygen species, and lipid metabolites.[5,8,9]

Lidocaine has various inhibitory effects on neutrophil function, including chemotaxis[10] and superoxide anion (O_2^-) release.[10,11] The drug has been shown to attenuate acute lung injury induced by endotoxin[12] or hyperoxia,[13] probably by inhibiting the function of neutrophils. Lidocaine is also likely to inhibit eosinophil-active cytokines[14] and the expression of cellular adhesion molecules on neutrophils.[15] Thus lidocaine may be able to prevent or attenuate acute lung injury induced by acid aspiration.

To test this hypothesis, we assessed the effect of intravenous treatment with lidocaine on hydrochloric acid (HCl)-induced lung injury.

Materials and Methods

Animal Preparation and Protocol

This experiment was conducted according to the guidelines of the animal care review board of Kobe University School of Medicine.

Japanese White rabbits (n = 56; weight, 2.2-2.6 kg)

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were anesthetized with 15 mg/kg ketamine injected intravenously through a catheter inserted into an ear vein and were intubated with a 3.5-mm endotracheal tube through a tracheotomy. An arterial catheter was placed through a cutdown in the right femoral artery to monitor blood pressure and take samples for blood gas analysis and assay. The animals were paralyzed with pancuronium bromide, and anesthesia was maintained with a continuous infusion of ketamine (10 mg $\cdot kg^{-1} \cdot h^{-1}$). The lungs of the animals were mechanically ventilated using an infant ventilator (model IV100B; Sechrist, Anaheim, CA) with an inspired oxygen concentration of 100%. The initial tidal volume was set to 10 ml/kg (measured by pneumotachograph) and positive end-expiratory pressure was 5 cm H_2O. The respiratory rate was controlled to produce an initial arterial carbon dioxide tension (Pa_{CO_2}) of 32–38 mmHg. Central venous pressure was also monitored using a catheter inserted into the femoral vein. The animals were placed on a heating pad under a radiant heat lamp so that the body temperature could be kept at 37.8–40.2°C at the esophagus. Lactated Ringer's solution was administered intravenously at a rate of 8 $ml \cdot kg^{-1} \cdot h^{-1}$.

After 15 min of stabilization, baseline values of lung mechanics and hemodynamics were measured, and the arterial blood sample was taken to determine arterial oxygen tension (Pa_{O_2}), peripheral leukocyte counts, and plasma cytokine concentrations.

Study 1. Twenty-eight rabbits were randomly assigned to one of four groups (n = 7 each). The rabbits in the saline-lidocaine and HCl-lidocaine groups received bolus intravenous injections of 2 mg/kg lidocaine followed by continuous intravenous infusion of lidocaine at a rate of 2 $mg \cdot kg^{-1} \cdot h^{-1}$. Animals in the saline-saline and HCl-saline groups received the same volume of saline (injection and infusion). Ten minutes after pretreatment with lidocaine or saline, the rabbits in the HCl-saline and HCl-lidocaine groups received 3 ml/kg 0.1 N HCl intratracheally. The animals in the saline-saline and saline-lidocaine groups received intratracheal saline (3 ml/kg).

Study 2. Rabbits (4 groups, n = 7 each) received HCl (0.1 N, 3 ml/kg) intratracheally. Saline was administered 10 min before injury in the saline group. Administration of lidocaine was started 10 min before, 10 min after, or 30 min after acid aspiration in the lidocaine-pre, lidocaine-10 min, and lidocaine-30 min groups, respectively. The rationale for the dose of intravenous lidocaine treatment was based on results of past experiments.[12,13,16,17]

Data on the hemodynamics and lung mechanics and blood sampling for arterial gas analysis and leukocyte counts were obtained every hour after HCl or saline intratracheal instillation. In all groups, arterial blood was drawn for the cytokine assay at 0, 2, 4, and 6 h, and the plasma was separated and stored at −70°C until assay. Plasma concentrations of interleukin-6 (IL-6) and IL-8 were measured by enzyme immunoassay (Amersham, Buckinghamshire, UK).

In groups receiving lidocaine, arterial blood samples were obtained 1, 2, 4, and 6 h after intratracheal instillation of HCl or saline. Plasma lidocaine concentrations were determined by fluorescence polarization immunoassay (TDX system, Abbott, North Chicago, IL).

Assessment of Acute Lung Injury

Analysis of Blood Samples and Lung Mechanics. Arterial blood gases (Pa_{O_2}, Pa_{CO_2}, and *p*H) were analyzed using a blood gas analyzer (ABL2 Radiometer, Copenhagen, Denmark), and the number of peripheral leukocytes was measured using a Coulter counter (Sysmex K-1000; Toa Iyou Denshi, Kobe, Japan). Lung mechanics were measured by the passive expiratory flow-volume technique.[18] Air flow was measured with a Flemish 00 pneumotachograph and a differential pressure transducer (model MP045; Validyne Engineering, Northbridge, CA). Airway pressure was measured at the proximal end of the pneumotachometer with a semiconductor pressure transducer (model P-300 501G; Copal Electronics, Tokyo, Japan). The volume was determined for each breath by digital integration of airflow using a respiration monitor (Aivision, Tokyo, Japan). The compliance and resistance of the total respiratory system were calculated using a personal computer (PC9801 VM11; NEC, Tokyo, Japan).

At the end of the experiment (6 h after intratracheal HCl or saline infusion), the thorax was opened and blood (15 ml) was drawn into a heparinized syringe (20 U/ml) from the pulmonary artery for the chemiluminescence assay. Blood sampling was completed before the overdose injection of thiamylal by which the rabbits were killed. The heart and lungs were removed *en bloc* by observers blinded to the nature of the experiments.

Analysis of Bronchoalveolar Lavage Fluid. Through the right mainstem bronchus of the removed lung, 35 ml saline with EDTA-2Na at 4°C was slowly infused and withdrawn. This procedure was repeated three times. Indomethacin was added to the bronchoalveolar lavage fluid (BALF) to inhibit the further metabolism of arachidonic acid to prostaglandins during analy-

sis. The BALF was analyzed for cell counts and differentiation. A cytocentrifuged preparation (Cytospin 2; Shandon Southern Products, Pittsburgh, PA) of the BALF was stained with Wright-Giemsa for cell differentiation. The cells present in the fluid were counted using the Sysmex K-1000 counter according to the Bürker-Türk method. The fluid was centrifuged at 250*g* at 4°C for 10 min to remove the cells. The cell-free supernatant was divided into several aliquots and stored at −70°C until the assay. The following substances, metabolites, and mediators in the BALF were measured: Albumin concentrations were determined by nephelometry with immunoglobulin G fraction of goat anti-rabbit albumin (Cappel, Durham, NC); concentrations of IL-6 and IL-8 were measured by enzyme immunoassay (Amersham); and concentration of thromboxane A_2 and prostacyclin were quantified by radioimmunoassay (Amersham) as thromboxane B_2 and 6-keto prostaglandin F1α, the stable metabolites, respectively.

Wet-to-Dry Weight Ratio of the Lung. The left upper lobe of each rabbit was weighed and then dried to a constant weight at 60°C for 24 h in an oven. The ratio of wet weight to dry weight was calculated to assess tissue edema.

Chemiluminescence Assay

Reagents. Cypridina luciferin analog (2-methyl-6-phenyl-3,7-dihydroimidazo [1,2-a]-pyrazin-3-one), dimethyl sulfoxide, Hank's balanced salt solution (HBSS), Histopaque-1119, Histopaque-1077, N-formyl-L-methionyl-L-luecyl-L-phenylalanine (FMLP), and zymosan A were obtained from Sigma Chemical Company (St. Louis, MO). The cypridina luciferin analog was dissolved to 56 μg/ml in distilled water. Five milligrams FMLP was dissolved in 1.14 ml dimethyl sulfoxide. Just before use, the stored solution was diluted with 50% DMSO - 50% HBSS to 100 μM. Zymosan A was opsonized according to the method of Nishida *et al.*[19] with some modification. Briefly, zymosan A was suspended in HBSS at a concentration of 2 mg/ml and heated in a boiling water bath for 100 min, washed twice with HBSS, and opsonized with pooled serum in a shaking water bath for 30 min at 37°C. The opsonized zymosan was washed twice, resuspended in HBSS to a concentration of 20 mg/ml, and stored at −70°C until use.

Isolation of Neutrophils. Histopaque-1119, Histopaque 1077, and whole blood were layered and centrifuged at 700*g* for 30 min at room temperature. The layer containing granulocytes (at the interphase between Histopaque-1077 and Histopaque-1119) was transferred to another tube. The cells were washed in HBSS and centrifuged twice at 200*g* for 10 min. The resultant leukocytes were suspended to 1×10^7 cells/ml in HBSS and kept at 0°C for no more than 3 h before use. The cell analysis showed that >97% of the cells were neutrophils, and the trypan blue dye exclusion test confirmed that >95% of the cells were viable.

Measurement of Chemiluminescence. Measurement of chemiluminescence was based on the method established by Sugioka *et al.*[20] The incubation mixture contained 4×10^5 leukocytes, 20 μl FMLP, or 80 μl opsonized zymosan, 25 μl cypridina luciferin analog, and HBSS in a total volume of 2 ml. Cells and HBSS were preincubated for 3 min, and the reaction was initiated by the simultaneous addition of the other two components. Cypridina luciferin analog-dependent luminescence, which is thought to represent primarily superoxide anion production, was monitored using a luminescence reader (Lumicounter-1000; Nichion, Chiba, Japan). During luminescence measurement, the incubation mixture was agitated at 37°C in the luminescence reader. Ketamine used as an anesthetic in the current study is thought to have no effect on superoxide anion production by neutrophils at doses used in clinical settings.[21]

Histopathologic Examination

Immediately after the rabbits were killed, the left lower lobe was fixed by instillation of 10% formaldehyde solution through the left lower bronchus at 20 cm H_2O. The specimens were embedded in paraffin wax, stained with hematoxylin and eosin, and examined under a light microscope. Acute lung injury was scored by a blinded observer according to the following four items: (1) alveolar congestion, (2) hemorrhage, (3) infiltration or aggregation of neutrophils in airspace or the vessel wall, and (4) thickness of the alveolar wall/hyaline membrane formation. Each item was graded according to a five-point scale: 0 = minimal (little) damage, 1+ = mild damage, 2+ = moderate damage, 3+ = severe damage, and 4+ = maximal damage.[12]

Statistics

The lung injury score data are given as median (range), whereas the other data are expressed as mean ± SD. Parametric data were analyzed using a one-way analysis of variance with the Tukey-Kramer test for between-group comparisons at each treatment interval and paired *t* tests for comparisons within groups. The lung

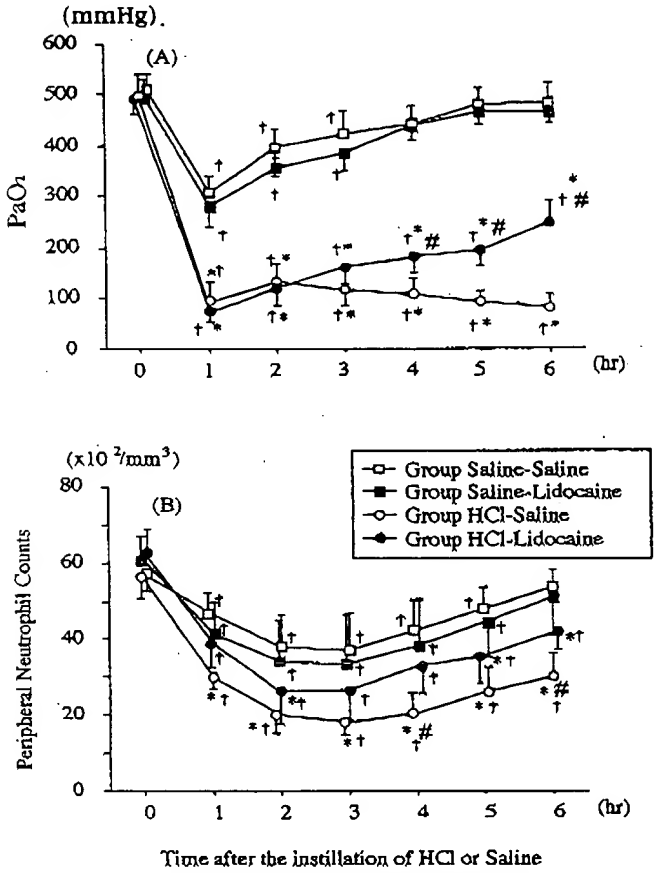

Fig. 1. Changes in (*A*) partial oxygen tension (Pa_{O_2}) and (*B*) peripheral leukocyte count (study 1). Data are expressed as the mean ± SD of seven rabbits per group. †$P < 0.05$ *versus* basal values within groups; *$P < 0.05$ *versus* the saline-saline group; #$P < 0.05$ for the HCl-lidocaine group *versus* the HCl-saline group.

injury score was analyzed using the Kruskall-Wallis rank test. Probability values <0.05 were deemed significant.

Results

No rabbits died during the 6-h study period. In groups receiving lidocaine, plasma lidocaine concentrations were maintained between 1.2–2.5 μg/ml.

Changes in Oxygen Tension, Hemodynamics, and Peripheral Leukocyte Counts

As shown in figures 1 and 2, intratracheal instillation of HCl decreased Pa_{O_2} dramatically within 1 h. Thereafter, Pa_{O_2} in the HCl-saline group remained at a low level with gradual reduction until 6 h after HCl instillation. Lidocaine treatment failed to prevent the initial decrease in Pa_{O_2}. Lidocaine before and 10 min after instillation of HCl promoted partial recovery from deteriorated oxygenation, although the drugs given 30 min after insult were not effective. In contrast, Pa_{O_2} decreased transiently in rabbits receiving saline instillation, but it readily returned toward the basal value (fig. 1).

Although arterial pressure and heart rate increased in response to HCl or saline instillation, there was no difference among the groups at any point (data not shown). The central venous pressure did not differ among the groups.

Peripheral leukocyte counts gradually decreased with

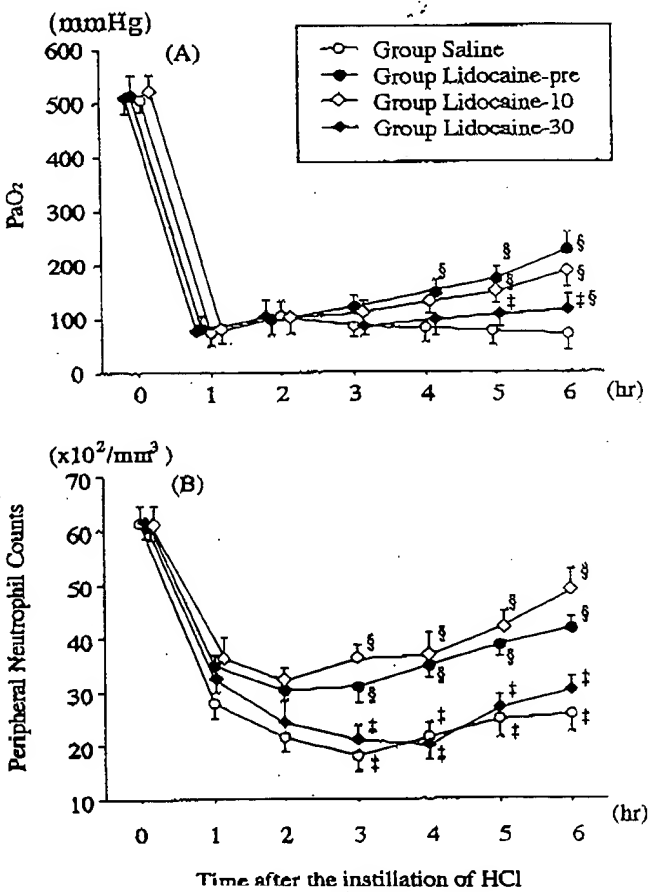

Fig. 2. Changes in (*A*) the partial oxgyen tension Pa_{O_2} and the (*B*) peripheral leukocyte count (study 2). Data are expressed as the mean ± SD of seven rabbits per group. §$P < 0.05$ *versus* the saline group; ‡$P < 0.05$ *versus* the lidocaine-pre group.

instillation of saline or HCl and reached the lowest level 3 h after administration (fig. 1B). The leukopenia was more severe in rabbits receiving HCl than in those that received saline. Lidocaine treatment before and 10 min after HCl aspiration slightly, but significantly, attenuated the peripheral leukopenia (fig. 2B).

Changes in Plasma Cytokine Levels

Plasma concentrations of IL-6 increased after intratracheal HCl instillation, with a peak occurring at 2 h, although plasma IL-8 concentrations remained unchanged in all of the groups. The plasma concentration of IL-6 was significantly decreased by intravenous lidocaine treatment before and 10 min after HCl administration. As with Pa_{O_2} changes, lidocaine given 30 min after HCl instillation was not effective against IL-6 elevation (figs. 3 and 4).

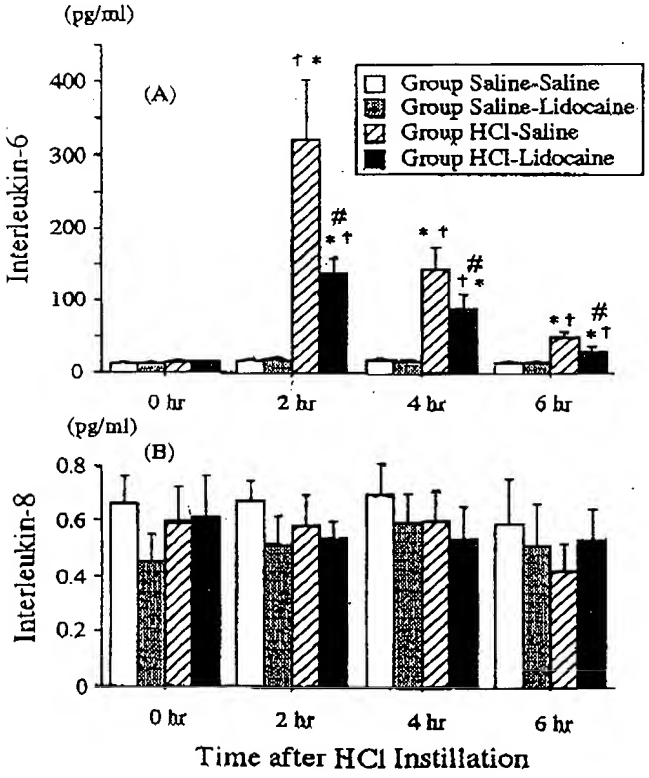

Fig. 3. Changes in plasma concentrations of (*A*) interleukin-6 and (*B*) interleukin-8 (study 1). Data are expressed as mean ± SD. †$P < 0.05$ *versus* basal values within groups; *$P < 0.05$ *versus* the saline-saline group; #$P < 0.05$ for the HCl-lidocaine group *versus* the HCl-saline group.

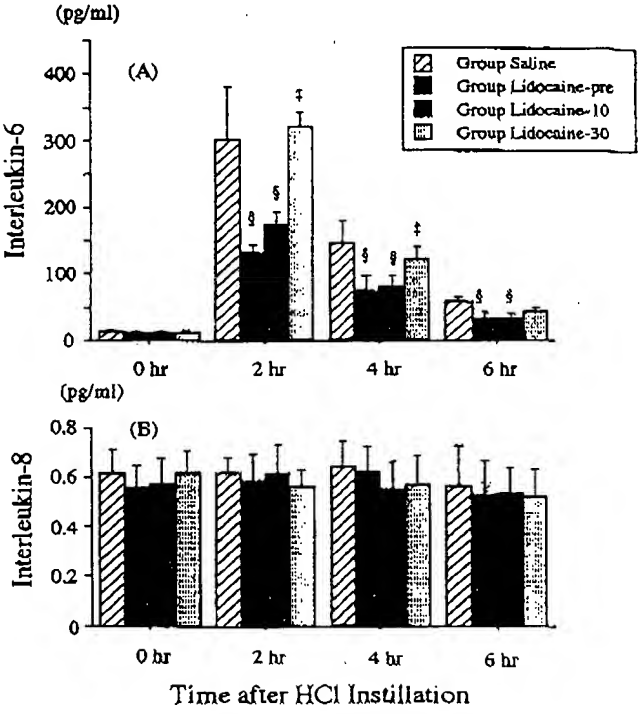

Fig. 4. Changes in plasma concentrations of (*A*) interleukin-6 and (*B*) interleukin-8 (study 2). Data are expressed as mean ± SD. §$P < 0.05$ *versus* the saline group; ‡$P < 0.05$ *versus* the lidocaine-pre group.

Lung Mechanics

Neither compliance nor resistance immediately after the start of mechanical ventilation was different among the four groups. Compliance immediately before the end of the study was lower in the HCl-treated rabbits than in the saline-instilled groups. Intratracheal instillation of HCl increased the resistance. Lidocaine prevented the reduction of compliance and the increase in resistance induced by HCl, although lidocaine given 30 min after insult was not significantly effective (figs. 5 and 6).

Analysis of Bronchoalveolar Lung Fluid and Lung Edema

Recovered volume of BALF in the groups was 84–90 ml (80–86% of infused saline) and was not different among the groups. The total number of leukocytes recovered in BALF was greater in the groups receiving HCl than in the intratracheal saline groups (table 1). As shown in table 2, the leukocyte counts were less in the lidocaine-pre and lidocaine-10 min groups than in the

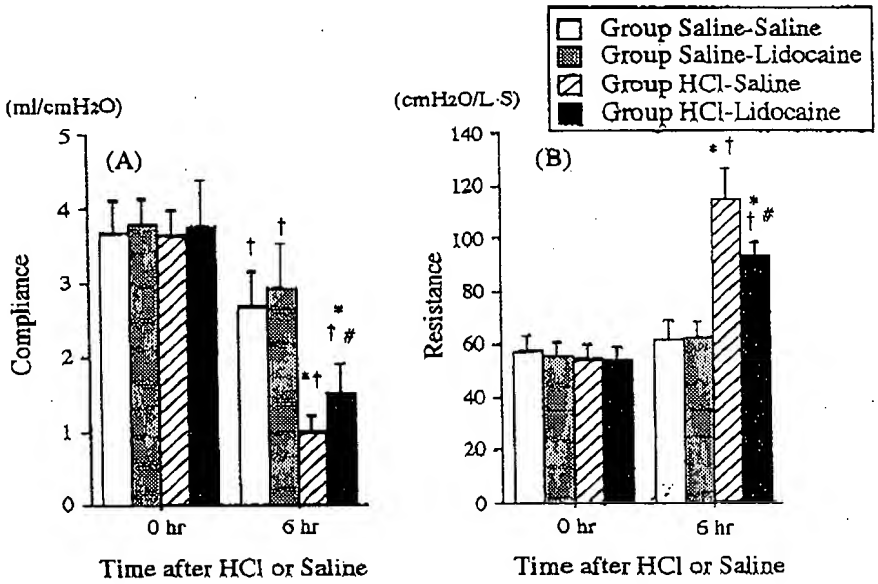

Fig. 5. Changes in lung mechanics: (*A*) compliance and (*B*) resistance (study 1). Data are expressed as mean ± SD. †*P* < 0.05 *versus* basal values within groups; **P* < 0.05 *versus* the saline-saline group; #*P* < 0.05 for the HCl-lidocaine group *versus* the HCl-saline group.

saline group. Differential counts revealed that leukocytes of the BALF in the groups receiving saline intratracheally were primarily macrophages. In the HCl-instilled groups, the number of polymorphonuclear cells was increased in the BALF. The polymorphonuclear cells-to-total leukocyte ratio was less in the rabbits treated with lidocaine, with the exception of the lidocaine-30 min group. Bronchoalveolar fluid concentrations of IL-6 and IL-8 increased in the rabbits receiving HCl. Lidocaine treatment started early was effective in decreasing cytokine production. The thromboxane B_2 concentrations in BALF were elevated in the HCl-saline

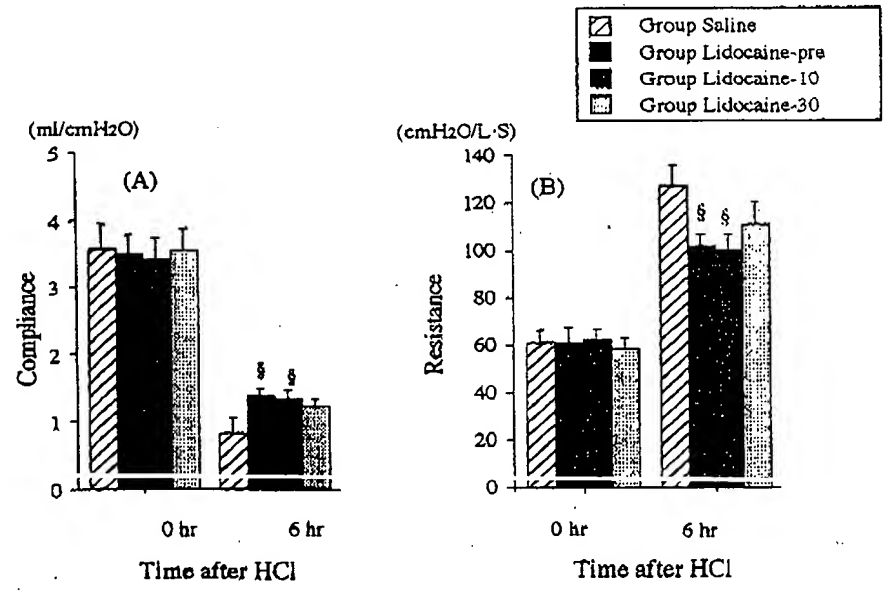

Fig. 6. Changes in lung mechanics: (*A*) compliance and (*B*) resistance (study 2). Data are expressed as mean ± SD. §*P* < 0.05 *versus* the saline group; ‡*P* < 0.05 *versus* the lidocaine-pre group.

Table 1. Analysis of Bronchoalveolar Lavage Fluid, Wet to Dry Weight Ratio, and Histological Examination (Study 1)

Variable	Saline–Saline	Saline–Lidocaine	HCl–Saline	HCl–Lidocaine
WBC (cells/mm³)	154 ± 44	146 ± 33	403 ± 101*	255 ± 73*†
PMN/total cells (%)	1 ± 1	1 ± 1	57 ± 9*	41 ± 10*
IL-6 (fmol/ml)	1 ± 1	2 ± 2	71 ± 25*	38 ± 10*†
IL-8 (fmol/ml)	2 ± 1	2 ± 2	43 ± 8*	14 ± 7*†
TxB_2 (pg/ml)	111 ± 71	117 ± 74	232 ± 95*	186 ± 87*
6-keto-$PGF_{1\alpha}$ (pg/ml)	215 ± 102	232 ± 88	235 ± 94	221 ± 112
Albumin (mg/dl)	1.8 ± 0.7	1.2 ± 0.3	6.4 ± 0.7*	5.1 ± 0.7*†
W/D ratio	4.6 ± 2.2	4.8 ± 2.3	6.5 ± 2.7*	5.5 ± 2.6*†
Acute lung injury score [median (range)]	0 (0–2)	0 (0–2)	14* (13–16)	10*† (7–14)

IL = interleukin; TxB_2 = thromboxane B_2; $PGF_{1\alpha}$ = prostaglandin $F_{1\alpha}$; WBC = white blood cells; PMN = polymorphonuclear neutrophils.

Values are mean ± SD.

* $P < 0.05$ *versus* Saline–Saline.

† $P < 0.05$, HCl–Lidocaine *versus* HCl–Saline.

group. Lidocaine treatment failed to attenuate the production of thromboxane B_2. We found no differences in 6-keto-prostaglandin F1α levels in the BALF among the groups. The lung wet-to-dry weight ratio was calculated as a measurement of lung edema. The ratio increased in the rabbits receiving HCl. Albumin concentrations in the supernatant of BALF were higher in the HCl-instilled rabbits than in saline-instilled rabbits. Lidocaine pretreatment attenuated the increase in the wet-to-dry weight ratio and albumin concentrations. Early post-treatment with lidocaine was as effective as pretreatment, but lidocaine given 30 min later was not.

Chemiluminescence

The cypridina luciferin analog-dependent chemiluminescence by neutrophils isolated from the pulmonary artery was significantly higher in the HCl-saline group than in the saline-saline group when stimulated by opsonized zymosan or FMLP (fig. 7). Chemiluminescence was attenuated in all of the lidocaine-treated groups.

Histopathologic Analysis

Light microscopic findings in the HCl-saline group included edema, ruptured and thickened alveolar walls, and the presence of inflammatory cells and erythrocytes in alveolar spaces. Alveoli also contained diffuse proteinaceous exudate. In contrast, these changes were less pronounced in the HCl-lidocaine group. The score in the HCl-saline group was greater than that in the HCl-lidocaine group (table 1) and in the lidocaine-10 min group (table 2).

Table 2. Analysis of Bronchoalveolar Lavage Fluid, Wet to Dry Weight Ratio, and Histological Examination (Study 2)

Variable	HCl–Saline	HCl–Lidocaine	HCl–Lidocaine 10 min	HCl–Lidocaine 30 min
WBC (cells/mm³)	413 ± 116	260 ± 70*	298 ± 92	334 ± 97
PMN/total cells (%)	55 ± 10	42 ± 9*	40 ± 10*	48 ± 9
IL-6 (fmol/ml)	73 ± 27	35 ± 10*	44 ± 18*	62 ± 22†
IL-8 (fmol/ml)	40 ± 8	15 ± 4*	21 ± 5*	37 ± 4†
TxB_2 (pg/ml)	243 ± 93	199 ± 86	186 ± 80	194 ± 85
6-keto-$PGF_{1\alpha}$ (pg/ml)	219 ± 97	205 ± 109	218 ± 81	239 ± 107
Albumin (mg/dl)	6.5 ± 0.8	4.9 ± 0.7*	5.0 ± 0.7*	6.2 ± 0.9†
W/D ratio	6.7 ± 2.5	5.4 ± 2.5*	5.6 ± 2.4*	6.3 ± 2.9†
Acute lung injury score [median (range)]	14 (13–16)	10* (7–13)	11* (8–14)	14† (11–16)

IL = interleukin; TxB_2 = thromboxane B_2; $PGF_{1\alpha}$ = prostaglandin $F_{1\alpha}$; WBC = white blood cells; PMN = polymorphonuclear neutrophils.

* $P < 0.05$ *versus* HCl–Saline.

† $P < 0.05$ *versus* HCl–Lidocaine.

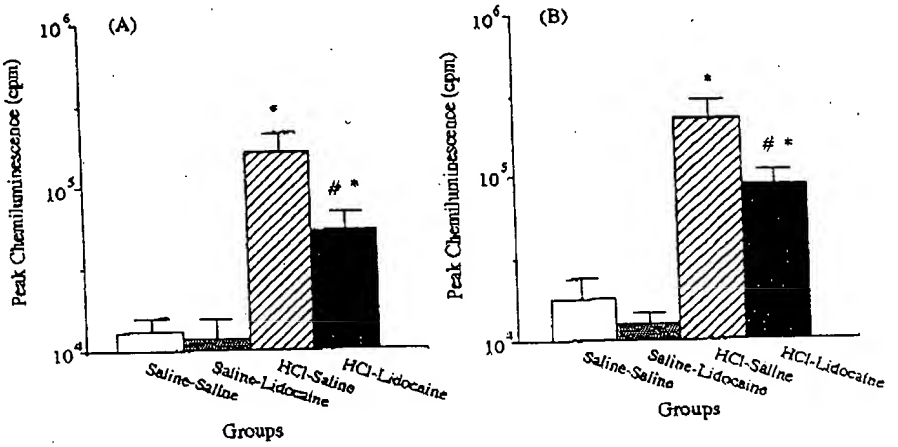

Fig. 7. Cypridina luciferin analog-dependent chemiluminescence (peak) by neutrophils isolated from the pulmonary artery (study 1). Data are expressed as mean ± SD. (*A*) Opsonized zymosan-stimulated chemiluminescence, (*B*) N-formyl-L-methionyl-L-luecyl-L-phenylalanine–stimulated chemiluminescence. *$P < 0.05$ *versus* the saline-saline group; #$P < 0.05$ for the HCl-lidocaine group *versus* the HCl-saline group.

Discussion

The current study showed that lidocaine pretreatment promoted the recovery of Pa_{O_2} after HCl instillation, although the drug did not prevent the initial decrease in Pa_{O_2}. This alleviating effect on lung injury by lidocaine was accompanied by improved lung mechanics and the decrease of cytokines in BALF, the albumin concentration in BALF, and sequestration of neutrophils into the lung. The efficacy of post-treatment with lidocaine depended on the timing of administration: 10 min after the insult was effective, but 30 min after was not.

Hydrochloric acid instillation caused lung injury by two different pathways according to the time course: the initial phase and the late phase.[2] The initial phase may result from a physiochemical process or be mediated by capsaicin-sensitive afferent nerves.[2] Tachykinins (substance P, neurokinin A, and neuropeptide K) released from sensory nerves in the lung after irritation with capsaicin are active in inducing airway mucosal edema.[22] Although lidocaine inhibits capsaicin-sensitive nerve activity[23] and tachykinin-mediated neurotransmission,[24] the drug failed to attenuate the initial phase of lung injury characterized by the dramatic decrease in Pa_{O_2}.

The late phase of the injury, which pathologically mimics acute respiratory distress syndrome, is mediated by neutrophils and is consistent with an acute inflammatory response.[2] In this pathway, chemotaxins, including IL-8 and thromboxane A_2, promote the sequestration of neutrophils in the lung.[7,25] To induce neutrophil migration into acid-exposed areas, it is essential for neutrophils to adhere to the microvascular endothelium.

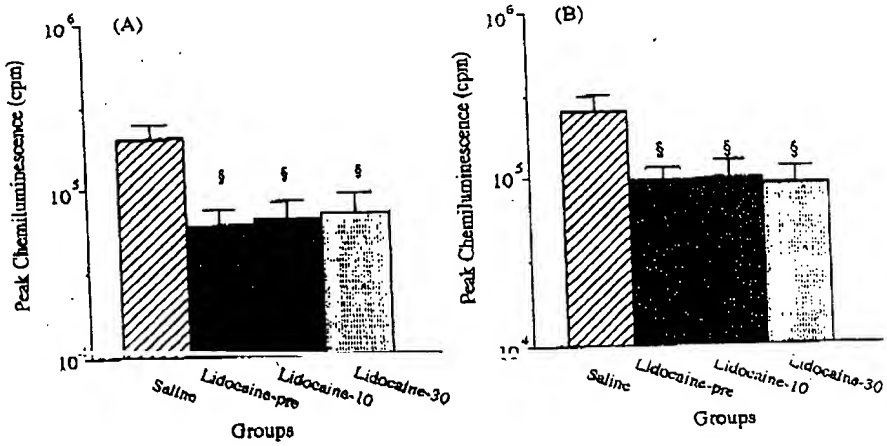

Fig. 8. Cypridina luciferin analog-dependent chemiluminescence (peak) by neutrophils isolated from the pulmonary artery (study 2). Data are expressed as mean ± SD. (*A*) Opsonized zymosan-stimulated chemiluminescence, (*B*) N-formyl-L-methionyl-L-luecyl-L-phenylalanine–stimulated chemiluminescence. §$P < 0.05$ *versus* the saline group.

The intercellular adhesion molecule-1 and lymphocyte-function associated antigen-1 pathway is involved in the pathogenesis of acid aspiration lung injury.[6] We have shown that lidocaine attenuated the late phase of the pathologic, physiologic, and biochemical lung injury induced by HCl. Lidocaine inhibits chemotaxis,[10] adherence of neutrophils to endothelial monolayers,[16] and FMLP-induced Mac-1 upregulation on neutrophils.[15] These beneficial effects of the drug may have contributed to our current findings.

We have shown that intratracheal HCl caused severe pulmonary edema because of the hyperpermeability of endothelium assessed by increases in the lung wet-to-dry weight ratio and albumin concentrations in BALF. Lidocaine successfully reduced superoxide anion production by neutrophils isolated from rabbits receiving HCl. We believe that lidocaine lessened endothelial damage, in part by reducing superoxide anion release from neutrophils, and consequently attenuated pulmonary edema. This ability of lidocaine was observed in our previous studies using other experimental acute respiratory distress syndrome models.[12,13] Further, superoxide anion indirectly contributes to edematous lung injury by inactivating antiproteases.[26] Although superoxide anion production at 6 h was suppressed in the HCl-lidocaine-30 min group, hyperpermeability was not attenuated. The early activation of neutrophils may be critical for the attenuation of endothelial damage leading to pulmonary edema. Lidocaine has been shown to decrease the increases in pulmonary vascular protein leak induced by thiourea through a mechanism that is independent of the effects on neutrophil oxygen metabolite-dependent toxicity, although the details are not understood.[27] This mechanism may also be responsible for the attenuation of acid instillation-induced pulmonary edema with lidocaine.

Thromboxane A_2 causes severe pulmonary hypertension as a result of pulmonary vasoconstriction, which enhances pulmonary edema.[28] In the current study, the thromboxane A_2 metabolites in BALF induced by HCl instillation did not decrease with lidocaine treatment. Although lidocaine is reported to increase production of prostacyclin from endothelium,[29] the metabolite of prostacyclin in the BALF was not changed either by acid instillation nor by lidocaine treatment.

High concentrations of IL-6[30] and IL-8[31] are correlated with poor outcome in patients with acute respiratory distress syndrome. In the current study, intratracheal HCl increased IL-8 concentrations and neutrophil counts in BALF and decreased the peripheral leukocyte counts. Lidocaine successfully decreased the secretion of IL-8 in the lung after HCl instillation, probably leading to less neutrophil sequestration in the lung, although the precise mechanism is unknown. Plasma IL-8 concentrations did not increase after HCl instillation in our study. The high concentrations of IL-8 in BALF coupled with the low concentrations of IL-8 in plasma suggest that the lung was the primary source of IL-8 in the acid aspiration-induced lung injury. This phenomenon is similar to the findings in a clinical study of sepsis-induced acute lung injury.[32] In our study, lidocaine before and early after treatment suppressed elevation of IL-6 in plasma and IL-8 in BALF. We propose two hypotheses by which lidocaine successfully attenuated the cytokine production. One is that lidocaine directly suppressed cytokine production from many kinds of cells, including macrophages, alveolar epithelium, and endothelium. There has been no report confirming that lidocaine attenuates production of proinflammatory cytokines. The second hypothesis is that lidocaine attenuated the inflammatory response, resulting in less production of cytokines. IL-6 is secreted from the most severely affected organs, as the higher concentration of IL-6 was measured in the drainage vein of the injured organ (lung or liver) than in the peripheral venous blood.[33] Lidocaine might decrease cytokine production by attenuating the inflammatory response.

In conclusion, pre- or early post-treatment with lidocaine attenuated the late phase of acid instillation-induced lung injury in rabbits, probably by inhibiting the sequestration and activation of neutrophils. Lidocaine, which has been used extensively in clinical practice, is not costly, has a good safety record, and is easily obtained. These advantages lead us to conclude that the results of the current study may be a basis for a clinical trial.

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Does Early Posttreatment with Lidocaine Attenuate Endotoxin-induced Acute Lung Injury in Rabbits?

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Background: It is well known that endotoxin causes acute lung injury, resulting in adult respiratory distress syndrome. Lidocaine pretreatment has recently been shown to attenuate endotoxin-induced lung injury in rabbits. The aim of the current study was to determine whether early postinjury treatment with intravenous lidocaine could attenuate acute lung injury induced by endotoxin in rabbits.

Methods: Thirty-two male anesthetized rabbits were randomly assigned to receive one of four treatments (n = 8 for each group): infusion of saline (group S-S), infusion of saline with lidocaine treatment (group S-L), infusion of *Escherichia coli* endotoxin (100 $\mu g \cdot kg^{-1}$ over a 60-min period) without lidocaine treatment (group E-S), or infusion of endotoxin with lidocaine treatment (group E-L). Ten minutes after the end of infusion of endotoxin (groups E-L and E-S) or saline (groups S-S and S-L), the animals received a bolus injection followed by continuous infusion of lidocaine (2 $mg \cdot kg^{-1}$ + 2 $mg \cdot kg^{-1} \cdot h^{-1}$ in groups S-L and E-L) or saline (groups S-S and E-S). The rabbits' lungs were ventilated with 40% O_2. Hemodynamics, peripheral leukocyte and platelet counts, and arterial O_2 tension (Pa_{O_2}) were recorded during the ventilation period (6 h). After the observation, lung mechanics; the cell fraction of bronchoalveolar lavage fluid (BALF); and concentrations of activated complement components C3a and C5a, cytokines, and arachidonic acid metabolites in BALF were measured and analyzed. The ratio of lung wet weight to dry weight (W/D weight ratio) and albumin concentrations in BALF were analyzed as indexes of pulmonary edema. The *Cypridina* luciferin analogue–dependent chemiluminescence (representing O_2^- production) by neutrophils isolated from the pulmonary artery and light-microscopic findings of the lung were compared among the four groups.

Results: Endotoxin caused decreases in peripheral leukocyte and platelet counts, lung compliance, and Pa_{O_2}. It caused increases in lung W/D weight ratio; polymorphonuclear cell counts in BALF; and albumin, C3a, C5a, tumor necrosis factor-α, interleukin (IL)-1β, IL-6, IL-8, and thromboxane B_2 concentrations in BALF. Lidocaine attenuated the changes in W/D weight ratio and morphologic lung damage. The change in compliance, decrease in Pa_{O_2}, and albumin concentrations in BALF were slightly but significantly less in rabbits receiving lidocaine after injury. The *Cypridina* luciferin analogue–dependent chemiluminescence by neutrophils was greater in rabbits receiving endotoxin without lidocaine than in those receiving endotoxin with lidocaine.

Conclusions: These results indicate that early treatment with lidocaine attenuates endotoxin-induced lung edema in rabbits without affecting chemical mediators in BALF. However, the improvement is slight and likely to be of little clinical significance. (Key words: Anesthetics, local: lidocaine. Immune response: neutrophils; superoxide anions. Lung(s): edema; lavage; respiratory distress syndrome.)

INTRAVENOUS infusion of *Escherichia coli* endotoxin causes acute lung injury and alterations in pulmonary physiologic processes similar to those that occur in septicemia in humans.[1,2] There have been a variety of experimental approaches to the prevention of acute lung injury.[3–5] We have shown that pretreatment with lidocaine significantly attenuated endotoxin-induced acute lung injury in rabbits.[6] Lidocaine effectively treats endotoxin-induced lung injury by attenuating O_2^- production by neutrophils, which is the final step of lung injury, without affecting the secretion of cytokines. In the current study, we investigated whether lidocaine would be effective when administered shortly after the onset of a gram-negative septic insult (postinjury treatment). We hypothesized that lidocaine administered soon after injury also may be effective in reducing endotoxin-induced lung injury by attenuation of neutrophil activation. In our previous study, we demonstrated severe peripheral neutropenia and pulmonary hypertension in rabbits within 60 min after the start of infusion of endotoxin.[6] Thus, we chose 10 min after administering endotoxin (over a 60-min period) as an

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appropriate time both after septic insult and before deterioration of oxygenation to test the efficacy of lidocaine.

Materials and Methods

Animal Preparation and Protocol

This study was conducted according to the guidelines of the animal care review board of Kobe University School of Medicine. Thirty-two male Japanese white rabbits weighing 2.0–2.4 kg were used in this study and randomly divided into four groups (n = 8 for each group) in a blinded manner as follows: rabbits in group S-S received saline alone without lidocaine posttreatment; group S-L received saline with lidocaine posttreatment; group E-S received endotoxin from *E. coli* (055:B5 from the same lot, Difco, Detroit, MI), without lidocaine posttreatment; and group E-L received endotoxin followed by lidocaine (fig. 1).

After the rabbits were sedated with 4 $mg \cdot kg^{-1}$ ketamine, tracheostomy was performed aseptically, and a 3.5-mm noncuffed endotracheal tube was inserted and tied in place. Anesthesia was maintained with continuous infusion of ketamine at a rate of 4 $mg \cdot kg^{-1} \cdot h^{-1}$. The lungs of the rabbits were ventilated with an infant ventilator (IV100B, Sechrist, Anaheim, CA) at an inspired O_2 concentration of 40%. Tidal volume was set to 10 $ml \cdot kg^{-1}$ measured by pneumotachograph. Respiratory rate was adjusted to produce an initial arterial CO_2 tension (Pa_{CO_2}) of 35–42 mmHg; Pa_{CO_2} was maintained at less than 50 mmHg throughout the study period.

Via femoral cutdown, a catheter was placed in the distal aorta to monitor arterial pressure and to take samples for blood gas analysis. Pulmonary arterial pressure was continuously monitored with a pulmonary artery catheter (3-French, Baxter, Chicago, IL) inserted through the right internal jugular vein. Central venous pressure was also monitored with a catheter inserted through the femoral vein. The animals were placed on a heating pad under a radiant heat lamp so that the body temperature could be kept at 37.7–40.3°C. Lactated Ringer's solution was intravenously administered at a rate of 8 $ml \cdot kg^{-1} \cdot h^{-1}$.

Immediately after the baseline measurement of lung mechanics, hemodynamics, peripheral leukocyte and platelet counts, and arterial blood gas analysis, groups S-S and S-L received infusion of saline for 60 min. Rabbits in groups E-L and E-S received endotoxin 100 $\mu g \cdot kg^{-1}$ over a 60-min period, with or without lidocaine treatment. Groups E-L and S-L received a bolus of lidocaine 2 $mg \cdot kg^{-1}$ (Fujisawa, Osaka, Japan), 10 min after the end of administration of endotoxin (E-L) or saline (S-L), followed by continuous infusion of lidocaine at a rate of 2 $mg \cdot kg^{-1} \cdot h^{-1}$ until the rabbits were killed. This infusion rate of lidocaine was also used in our previous study.[6]

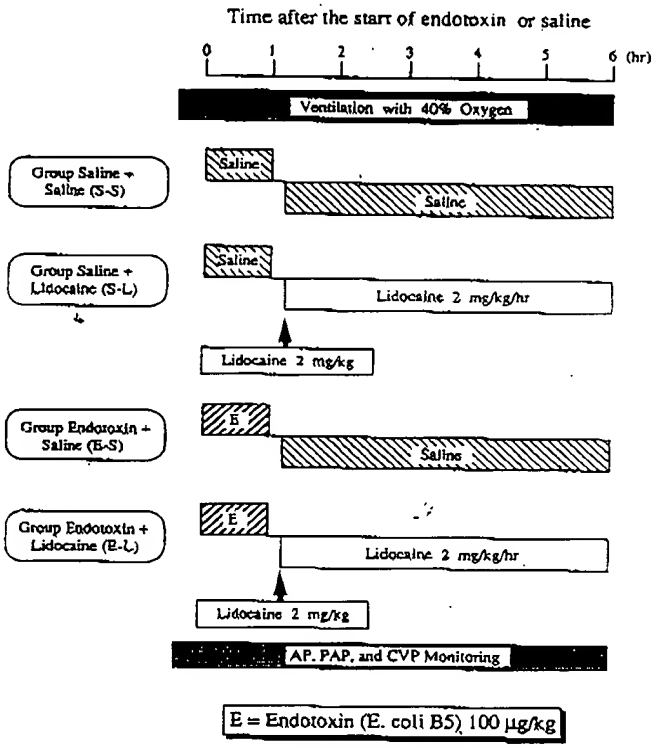

Fig. 1. Study protocol. AP = arterial blood pressure; PAP = pulmonary arterial pressure; CVP = central venous pressure.

All rabbits were killed 6 h after the start of endotoxin treatment by injection of thiamylal. In groups S-L and E-L, arterial blood samples were obtained at 0, 0.5, 1, 2, 3, and 4 h after the start of administration of lidocaine and at the time of killing to measure plasma concentrations of the drug using fluorescence polarization immunoassay (TDX system, Abbott, North Chicago, IL).

Estimation of Acute Lung Injury

Measurement of Lung Mechanics. During each experimental period, we obtained arterial blood specimens for analyzing arterial O_2 tension (Pa_{O_2}), Pa_{CO_2},

and *p*H using an automatic blood gas and electrolyte analyzer (ABL2, Radiometer, Copenhagen, Denmark) and for counting the number of peripheral leukocytes and platelets (counter, Coulter Electronics, Harkenden, United Kingdom). Immediately after the start of mechanical ventilation (before infusion of saline or endotoxin), at the end of administration of endotoxin, and immediately before the rabbits were killed (after the period of observation), lung mechanics were measured by the passive expiratory flow–volume technique as described by LeSouef *et al.*[7] The air flow was measured with a Fleish 00 pneumotachograph and a differential pressure transducer (MP-45, Validyne Engineering, Northridge, CA). Airway pressure was measured at the proximal end of the pneumotachometer with a semiconductor pressure transducer (P-300 501G, Copal Electronics, Tokyo, Japan). The volume was measured for each breath by digital integration of air flow using a respiration monitor (Aivision, Tokyo, Japan) and a personal computer (PC9801 VM11, NEC, Tokyo, Japan). The lungs were inflated and the air flow was interrupted at 20 cmH_2O. The occlusion was rapidly released after airway pressure reached a plateau. Compliance and resistance of the total respiratory system were then calculated by means of the personal computer.

At the end of the experiment, after the thorax was opened, blood (15 ml) was drawn into a heparinized syringe (20 $U \cdot ml^{-1}$) from the pulmonary artery for chemiluminescence assay (see below). The rabbits were killed by administration of thiamylal after sampling of blood. The heart and lungs were then removed *en bloc* by observers blinded to the nature of experiment.

Ratio of Lung Wet Weight to Dry Weight. The left upper lobe was weighed and then dried to constant weight at 60°C for 24 h in an oven. The ratio of wet weight to dry weight (W/D weight ratio) was calculated to assess tissue edema.

Preparation of Bronchoalveolar Lavage Fluid and Measurements. Through the right mainstem bronchus 40 ml saline with ethylenediamine tetraacetic acid–2Na at 4°C was slowly infused and withdrawn. This procedure was repeated five times. Indomethacin was added to the bronchoalveolar lavage fluid (BALF) to inhibit further metabolism of arachidonic acid to prostaglandins during analysis. The BALF was analyzed for cell count and cell differentiation. A cytocentrifuged preparation (Cytospin 2, Shandon Southern Products, United Kingdom) of the BALF was stained with Wright-Giemsa for cell differentiation. The cells present in the fluid were counted with the Coulter counter and the Bürker-Türk method.[8]

The fluid was centrifuged at 250*g* at 4°C for 10 min to remove the cells. The cell-free supernatant was divided into several aliquots and stored at −70°C until assayed. The following substances, metabolites, and mediators in the BALF were then measured. (1) The activated complement components C3a and C5a were quantified by radioimmunoassay (Amersham, Bucks, UK). (2) Albumin concentrations were measured by nephelometry with immunoglobulin G fraction of goat antirabbit albumin (Cappel, PA). (3) Concentrations of tumor necrosis factor α (TNFα), interleukin (IL)–1β, IL-6, and IL-8 were measured by enzyme immunoassay (Amersham, Bucks, United Kingdom). (4) Concentrations of thromboxane A_2 (TxA_2) and prostacyclin were quantified by radioimmunoassay (Amersham) as thromboxane B_2 (TxB_2) and 6-keto-prostaglandin $F_1\alpha$, the stable metabolites of TxA_2 and prostacyclin, respectively.

Chemiluminescence Assay. ***Reagents.*** *Cypridina* luciferin analogue (CLA) (2-methyl-6-phenyl-3,7-dihydroimidazo [1,2-a]-pyrazine-3-one), dimethyl sulfoxide, Hank's balanced salt solution (HBSS), Histopaque-1119, Histopaque-1077, N-formyl-L-methionyl-L-luecyl-L-phenylalanine (FMLP), and zymosan A were obtained from Sigma Chemical (St. Louis, MO).

The CLA was dissolved to 56 $\mu g \cdot ml^{-1}$ in distilled water. The solution was stored in 1-ml aliquots at −80°C. The CLA concentrations were based on E410 nm = 8900 $M^{-1} \cdot cm^{-1}$.[9] FMLP 5 mg was dissolved in 1.14 ml DSMO. The solution was stored at −80°C until the time of the assay. Just before use, the stored solution was diluted with 50% dimethyl sulfoxide–50% HBSS to 100 μM. Zymosan A was opsonized by the method of Nishida *et al.*[10] with modification. Zymosan A was suspended in HBSS at a concentration of 2 $mg \cdot ml^{-1}$ and heated in a boiling water bath for 100 min, washed twice with HBSS, and opsonized with pooled serum in a shaking water bath for 30 min at 37°C. The opsonized zymosan (OZ) was then washed twice, resuspended in HBSS to a concentration of 20 $mg \cdot ml^{-1}$, and stored at −80°C until use.

Isolation of Neutrophils. Histopaque-1119, Histopaque-1077, and whole blood were layered in a test tube and centrifuged at 700*g* for 30 min at room temperature. The layer containing granulocytes (at the interphase between Histopaque-1077 and Histopaque-1119) was transferred to another tube. The cells were washed in HBSS and centrifuged twice at 200*g* for 10

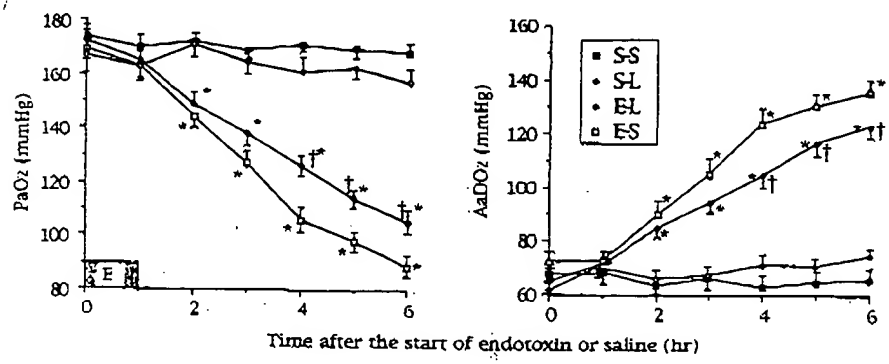

Fig. 2. Changes in arterial O_2 tension (Pa_{O_2}) and alveolar − arterial difference in O_2 tension (AaD_{O_2}) (mean ± SEM) in the three groups. E = intravenous infusion of *Escherichia coli* endotoxin (100 μg/kg) or saline; S-S = group receiving saline; S-L = group receiving saline and lidocaine; E-S = group receiving endotoxin and no lidocaine; E-L = group receiving endotoxin and lidocaine. *$P < 0.05$ *versus* group S-S; †$P < 0.05$, group E-L *versus* group E-S.

min. The resultant leukocytes were suspended to 1×10^7 cells · ml^{-1} in HBSS and were kept at 0°C for no longer than 3 h before use. The cell analysis showed that more than 96% of the cells were neutrophils, and the trypan blue dye exclusion test confirmed that more than 95% of the cells were viable.

Measurement of Chemiluminescence. Measurement of chemiluminescence was made by the method of Sugioka *et al.*[11] The incubation mixture contained 4×10^5 white blood cells (WBC), 20 μl FMLP or 80 μl OZ, 50 μl 40 μM CLA, and HBSS to a total volume of 2 ml. Cells and HBSS were preincubated for 3 min and the reaction initiated by the simultaneous addition of the other two components. CLA-dependent luminescence, which is thought to reflect primarily O_2^- production, was monitored with a luminescence reader (Lumicounter-1000, Nichion, Chiba, Japan). During luminescence measurement, the incubation mixture was agitated at 37°C in the luminescence reader. Ketamine used as an anesthetic in the current study has been shown to have no effect on O_2^- production by neutrophils at doses used in the clinical setting.[12]

Histopathologic Examination

Shortly after the rabbits were killed (<5 min), the left lower lobe was fixed by instillation of 10% formaldehyde solution through the left lower bronchus at 20 cmH_2O. The specimens were embedded in paraffin wax, and stained with hematoxylin and eosin and examined under a light microscope. Lung injury was scored as 0 (minimal damage) to 4+ (maximal damage) according to combined assessments of alveolar congestion; hemorrhage and edema; infiltration or aggregation of neutrophils in air space or vessel wall; thickness of alveolar wall, and hyaline membrane formation by two observers unaware of the group assignment of the animal.

Statistical Analysis

Data except lung injury score are expressed as means ± SEM; data on lung injury score are given as medians and range. The degree of attenuation of lung injury by lidocaine was calculated from the following formula: percentage attenuation = $100 \times (b - c)/(b - a)$, where a = value in S-S group; b = value in E-S group; and c = value in E-L group. This value indicates the degree of efficacy of lidocaine treatment in each subject: 0% indicates that the mean scores for groups E-S and E-L were equal (no attenuation of lung injury by lidocaine), and 100% indicates that the mean scores for group E-L and S-S were equal (maximum attenuation). Statistical analysis was performed by repeated-measures analysis of variance for continuous variables, except for lung injury score, for which the Kruskall-Wallis rank test was used. $P < 0.05$ was deemed significant. When analysis of variance indicated a significant difference, Bonferroni's multiple-comparison test was used to determine which groups were significantly different from each other.

Results

Changes in Arterial O_2 Tension, Hemodynamics, and Peripheral Leukocyte and Platelet Counts

No rabbits died of endotoxemia. In groups S-L and E-L, plasma lidocaine concentrations were maintained between 1.2–2.3 μg · ml^{-1}. As shown in figure 2, Pa_{CO_2} in groups S-S and S-L remained at a level exceeding 150 mmHg, whereas Pa_{O_2} in group E-S gradually decreased to 89 mmHg during the experiment. In group E-L, however, the decrease in this parameter was attenuated maximally by 29% ($P < 0.05$). The values of Pa_{CO_2} in four groups were similar (38 to 40 mmHg) and gradually increased to 50 ± 1.2 and 52 ± 1.3 in

Table 1. Changes in Hemodynamics and Peripheral Leukocyte and Platelet Counts in the Four Groups

	Time after the Start of Endotoxin or Saline							
	0 h	0.5 h	1 h	2 h	3 h	4 h	5 h	6 h
MPAP (mmHg)								
S-S	19 ± 1	20 ± 1	19 ± 1	17 ± 1	19 ± 1	17 ± 1	20 ± 1	18 ± 1
S-L	18 ± 1	20 ± 1	18 ± 1	19 ± 1	18 ± 1	20 ± 1	18 ± 1	17 ± 1
E-S	17 ± 1	30 ± 1*	32 ± 1*	29 ± 1*	25 ± 1*	24 ± 1*	22 ± 2*	22 ± 1*
E-L	19 ± 1	32 ± 1*	33 ± 1*	28 ± 1*	23 ± 1*	22 ± 1*	20 ± 1*	19 ± 1*
Leukocytes (× 10^2 cells/mm^3)								
S-S	40 ± 4	38 ± 4	39 ± 5	40 ± 4	43 ± 3	37 ± 4	35 ± 3	39 ± 4
S-L	38 ± 4	37 ± 4	36 ± 6	40 ± 4	43 ± 3	37 ± 4	35 ± 3	39 ± 4
E-S	41 ± 4	16 ± 1*	9 ± 0.7*	5 ± 0.2*	5 ± 0.3*	6 ± 0.3*	8 ± 0.3*	9 ± 0.7*
E-L	42 ± 4	15 ± 1*	9 ± 0.7*	5 ± 0.2*	6 ± 0.3*	6 ± 0.3*	9 ± 0.3*	10 ± 0.7*
Platelets (× 10^4 cells/mm^3)								
S-S	29 ± 3	32 ± 3	30 ± 3	33 ± 3	29 ± 3	27 ± 3	29 ± 3	26 ± 3
S-L	31 ± 3	29 ± 3	28 ± 3	30 ± 3	29 ± 3	29 ± 3	28 ± 3	27 ± 3
E-S	30 ± 3	29 ± 3	26 ± 3	22 ± 3*	17 ± 3*	15 ± 3*	16 ± 3*	14 ± 3*
E-L	33 ± 3	31 ± 3	28 ± 3	23 ± 3*	19 ± 3*	20 ± 3*	19 ± 3*	20 ± 3*

Values are mean ± SEM.

MAP = mean arterial pressure; HR = heart rate; CVP = central venous pressure; MPAP = mean pulmonary arterial pressure.

* $P < 0.05$ *versus* group S-S, $P > 0.05$ for group E-L *versus* group E-S.

groups E-L and E-S, respectively. The alveolar − arterial difference in O_2 tension was increased in groups E-L and E-S as Pa_{O_2} decreased. Lidocaine attenuated the increase in the alveolar − arterial difference in O_2 tension maximally by 31% ($P < 0.05$). The heart rate, arterial blood pressure, or central venous pressure did not differ among the four groups at any point (data not shown). Infusion of endotoxin rapidly increased pulmonary arterial pressure, with a peak reached at the end of endotoxin infusion. Lidocaine posttreatment failed to attenuate the pulmonary hypertension (table 1). Peripheral blood leukocyte counts decreased with infusion of endotoxin, reached their nadir 1–2 h after the end of endotoxin infusion, and remained low during the experiment. Lidocaine posttreatment also failed to attenuate the decrease in leukocyte. Peripheral blood platelet counts decreased gradually in the endotoxin-treated group (E-S and E-L). No effect of lidocaine on platelet counts was observed.

Lung Mechanics

Neither compliance nor resistance immediately after the start of mechanical ventilation and at the end of endotoxin treatment was different among the four groups (table 2). Compliance 6 h after the start of treatment with endotoxin was greater in group E-L than in group E-S (attenuation 29%; $P < 0.05$). In contrast, resistance in group E-L 6 h after endotoxin was similar to that in group E-S (attenuation 27%).

Ratio of Lung Wet Weight to Dry Weight

The lung W/D weight ratio was calculated as a parameter of lung edema. The ratio increased in rabbits receiving endotoxin (E-S and E-L) compared with those

Table 2. Lung Mechanics before and after Endotoxin or Saline

	Time after the Start of Endotoxin or Saline		
	0 h	1 h	6 h
Compliance (ml/cmH_2O)			
Group S-S	2.92 ± 0.09	2.88 ± 0.10	2.81 ± 0.14
Group S-L	2.88 ± 0.08	2.93 ± 0.11	2.78 ± 0.13
Group E-S	2.94 ± 0.08	2.62 ± 0.11	1.75 ± 0.12*
Group E-L	2.87 ± 0.08	2.64 ± 0.09	2.06 ± 0.12*†[29]
Resistance ($cmH_2O \cdot L^{-1} \cdot s^{-1}$)			
Group S-S	97 ± 4	101 ± 5	102 ± 6
Group S-L	100 ± 4	98 ± 5	104 ± 7
Group E-S	95 ± 4	106 ± 6	125 ± 6*
Group E-L	101 ± 4	105 ± 5	119 ± 6*[27]

Values are mean ± SEM. The percent attenuation of the variables by lidocaine is shown in parentheses.

% attenuation = (group E-S − group E-L) × 100/(group E-S − group S-S).

* $P < 0.05$ *versus* group S-S.

† $P < 0.05$ for group E-L *versus* group E-S.

receiving saline (S-S and S-L) (table 3). Lidocaine posttreatment attenuated the increase in W/D weight ratio (attenuation 43%; $P < 0.05$).

Analysis of Bronchoalveolar Lavage Fluid

Recovery in BALF in the three groups was 83–89%, indicating no difference between the four groups. Table 3 shows that the total number of leukocytes recovered in BALF was significantly higher in groups E-S and E-L compared with that in group S-S. Leukocyte counts in BALF were not significantly different in groups E-L and E-S (attenuation 15%). Differential counts revealed that BALF leukocytes in group S-S were mostly macrophages. Polymorphonuclear cells accounted for 1% of the WBC in BALF obtained from group S-S. In contrast, the ratio of polymorphonuclear cells to total WBC increased to 12% in group E-S and 8% in group E-L ($P > 0.05$).

Albumin concentrations in the supernatant of BALF were higher in endotoxin-treated rabbits (E-S and E-L) than in the saline-treated rabbits (S-S and S-L). Lidocaine posttreatment significantly decreased the albumin concentrations in endotoxin-treated rabbits (E-L) (attenuation 37%). Table 3 indicates that the concentrations in BALF of C3a and C5a, which are known to be chemotactic factors, were similar in groups E-L and E-S. The BALF concentrations of cytokines significantly increased in endotoxin-treated rabbits (E-S and E-L). The TxB_2 concentration in BALF increased in group E-S compared with that in group S-S. Posttreatment with lidocaine failed to attenuate the increase. In contrast, there were no differences in BALF concentrations of 6-keto-prostaglandin $F_1\alpha$ among the four groups.

Chemiluminescence

The CLA-dependent chemiluminescence (representing O_2^- production) by neutrophils isolated from the pulmonary artery blood in group E-S was significantly higher compared with that in group S-S when stimulated by OZ or FMLP (table 4). Posttreatment with lidocaine attenuated the increase in chemiluminescence (attenuation 75% for OZ and 64% for FMLP).

Histopathologic Findings

The results of the grading of lung damage are summarized in table 5. The score for E-L group was statistically less than that for group E-S. Light-microscopic findings in group E-S included hemorrhage and edema, thickened alveolar septa, and the presence of inflammatory cells in alveolar spaces; these changes were slightly less pronounced in group E-L.

Discussion

In the current study, we have shown that early posttreatment with lidocaine slightly attenuated deterio-

Table 3. Wet/Dry Weight Ratio and Analysis of Bronchoalveolar Lavage Fluid (BALF)

Variable	Group				% Attenuation
	S-S	S-L	E-S	E-L	
Total white blood cells (cells/mm³)	196 ± 22	215 ± 24	513 ± 38*	455 ± 31*	18
% PMN	1 ± 0.1	1 ± 0.1	12 ± 3*	8 ± 2*	36
Wet/dry weight ratio	4.8 ± 0.1	4.9 ± 0.1	5.5 ± 0.1*	5.2 ± 0.1†	43
Concentrations in BALF					
Albumin (mg/dl)	1.3 ± 0.3	1.2 ± 0.2	9.2 ± 1.0*	6.3 ± 0.7*†	37
C3a (ng/dl)	25 ± 4	23 ± 4	52 ± 9*	43 ± 6*	33
C5a (ng/dl)	6 ± 1	5 ± 1	19 ± 4*	17 ± 5*	15
TNFα (fmol/ml)	1 ± 1	1 ± 1	21 ± 5*	18 ± 3*	15
IL-1β (fmol/ml)	1 ± 1	1 ± 1	19 ± 3*	15 ± 3*	22
IL-6 (fmol/ml)	3 ± 1	3 ± 1	76 ± 10*	57 ± 6*	26
IL-8 (fmol/ml)	3 ± 1	2 ± 1	48 ± 7*	31 ± 6*	38
T × B_2 (pg/ml)	115 ± 28	107 ± 25	214 ± 34*	196 ± 31*	18
6-keto-$PGF_{1\alpha}$ (pg/ml)	202 ± 38	228 ± 50	221 ± 42	205 ± 41	—

Values are mean ± SEM.

—: because 6-keto-$PGF_{1\alpha}$ is thought to be a beneficial mediator for attenuation of lung injury, percent attenuation is not calculated.

PMN = polymorphonuclear cell; IL = interleukin; T × B_2 = thromboxane B_2; $PGF_{1\alpha}$ = prostaglandin $F_{1\alpha}$; % attenuation = (group E-S − group E-L) × 100/(group E-S − group S-S).

* $P < 0.05$ *versus* group S-S.

† $P < 0.05$ for group E-L *versus* group E-S.

Table 4. *Cypridina* Luciferin Analogue-dependent Chemiluminescence (Peak) by Neutrophils Isolated from the Pulmonary Artery Blood

Group	Opsonized Zymosan -stimulated (× 10^6 cpm)	FMLP-stimulated (× 10^6 cpm)
S-S	2.1 ± 0.2	2.5 ± 0.2
S-L	1.3 ± 0.1*	1.7 ± 0.1*
E-S	4.1 ± 0.4*	6.7 ± 0.5*
E-L	2.6 ± 0.3† (75)	4.0 ± 0.4*† (64)

Values are mean ± SEM. The percent attenuation of the chemiluminescence by lidocaine shown in parentheses was calculated according to the following formula: % attenuation = (group E-S − group E-L) × 100/(group E-S − group S-S).

* $P < 0.05$ *versus* group S-S.

† $P < 0.05$ for group E-L *versus* group E-S.

ration of oxygenation in endotoxin-treated rabbits (E-S and E-L). Lidocaine posttreatment also attenuated endotoxin-induced pulmonary edema as assessed by W/D weight ratio. The drug was effective morphologically (assessed by lung injury score) and functionally (assessed by compliance) for the acute lung injury. In contrast, lidocaine posttreatment did not decrease the release of chemotaxins (C3a, C5a, TNFα, IL-1β, and IL-8 in BALF) in rabbits receiving endotoxin (E-L). There were no differences between the groups E-L and E-S in the percentage of alveolar neutrophils recovered in BALF and leukocyte counts in peripheral blood. These observations suggest that lidocaine posttreatment could not inhibit the release of the chemotaxins, resulting in failure to reduce accumulation of leukocytes in the lung.

The pulmonary arterial pressure increased in rabbits receiving endotoxin (E-S and E-L), peaking at the end of endotoxin treatment. The pulmonary hypertension continued until 3 h after the end of endotoxin treatment. Lidocaine had no effect on this increased pulmonary arterial pressure. No difference in TxB_2 concentrations in BALF was observed in endotoxin-treated groups with or without lidocaine (E-S or E-L). This failure of lidocaine to decrease TxB_2 concentrations may be responsible for failure of lidocaine to decrease of pulmonary artery pressure. Lidocaine posttreatment lessened the endotoxin-induced increase in BALF concentrations of albumin, which is an index of endothelial hyperpermeability leading to pulmonary edema. The beneficial effect of lidocaine on pulmonary edema, which is assessed by W/D weight ratio and pathologic changes, may be due to attenuation of vascular hyperpermeability. Successful use of lidocaine to reduce lung extravascular protein accumulation, as an index of endothelial hyperpermeability, in thiourea-induced lung injury has been reported.[13]

We began the lidocaine treatment 10 min after the end of endotoxin infusion. We chose this timing because significant pathophysiologic events (pulmonary hypertension and profound leukopenia) have already occurred at this time.[6] It is well known that pulmonary hypertension and neutropenia are initial events in endotoxin-induced lung injury.[14–16]

We have hypothesized that posttreatment with lidocaine attenuates lung injury by the suppression of activation of neutrophils, as does pretreatment. Thus we evaluated the effect of lidocaine on acute lung injury until approximately 5 h after starting treatment with lidocaine, focusing our attention on the period of activating neutrophils and macrophages. As in the control group of the current study, endotoxin causes increases in production and release of cytokines, O_2^-, thromboxanes, and complement components, resulting in accumulation of neutrophils in lung, deterioration of oxygenation, and increase in extravascular lung water within 6 h. Lung vascular permeability to proteins increased between 2 and 6 h after endotoxin injection.[17,18] Chemiluminescence response reveals that neutrophils in blood are activated early after endotoxin, followed by metabolic exhaustion with a minimal chemiluminescence response after 2 h. The response is augmented thereafter by new granulocytes liberated from the bone marrow.# Accumulation of neutrophils in affected tissue probably became peaked by 6 h after

Table 5. Mean Lung Injury Score (median (range))

Group	n	Lung Injury Score
S-S	8	0 (0)
S-L	8	0 (0)
E-S	8	3 (2–4)*
E-L	8	2 (1–4)*†

Values are medians (with range in percentages).

Scores: 0 = minimal (little) damage; 1+ = mild damage; 2+ = moderate damage; 3+ = severe damage; 4+ = maximal damage.

* $P < 0.05$ *versus* group S-S.

† $P < 0.05$ for group E-L *versus* group E-S.

Dwenger A, Regel G, Ellendorff B, Schweitzer G, Funck M, Limbrock H, Sturm JA, Tscherne H. Alveolar cell pattern and chemiluminescence response of blood neutrophils and alveolar macrophages in sheep after endotoxin infection. J Clin Chem Clin Biochem 28: 163–168, 1990.

endotoxin administration, because the WBC counts in peripheral blood have started increasing.

We previously reported that lidocaine administered before endotoxin reduced endotoxin-induced lung injury.[6] Lidocaine pretreatment attenuates the deterioration of pulmonary hypertension, peripheral leukopenia, oxygenation, lung compliance, W/D ratio, and WBC counts, percentage of polymorphonuclear cells in total WBC, albumin in BALF, and O_2^- production by neutrophils with stimulation. However, lidocaine posttreatment was effective only in oxygenation, lung edema (assessed by albumin concentration in BALF, W/D ratio, and morphologic examination), and lung compliance. Lidocaine attenuated chemiluminescence of neutrophils to the same degree regardless of the timing of administration (attenuation 65% and 83% [pretreatment] and 64% and 75% [posttreatment], FMLP- and OZ-stimulated chemiluminescence, respectively). This attenuation of O_2^- production, which is known to be a major factor involved in lung injury as a final step, is a putative mechanism for reducing lung edema by lidocaine in this study.

Lidocaine after endotoxin failed to suppress chemical mediators despite improved pulmonary hyperpermeability. Some mediators that concern lung injury induced by endotoxin were not measured in the current study (*e.g.*, protease derived from neutrophils, phospholipase A_2, platelet-activating factor [PAF], and endothelin). Protease from activated neutrophils and macrophages directly attacks lung matrix, causing destruction of lung structure. Lidocaine stabilizes the cell membrane to decrease the release of proteases from neutrophils or macrophages.[19] Phospholipase A_2 activated by endotoxin causes degradation of phospholipid to arachidonic acids, as substrates of eicosanoids. TxA_2, produced by endothelium, platelets, and pulmonary macrophages, causes early phase pulmonary hypertension lasting about 90 min after starting endotoxin infusion.[20,21] It was not affected by lidocaine posttreatment probably due to its prompt release. As the results of this study showed that the concentration of TxA_2 and 6-keto-prostaglandin $F_1\alpha$ concentration in BALF were similar in groups E and E-L, lidocaine posttreatment would not prevent activation of phospholipase A_2. PAF directly and indirectly increases pulmonary vascular resistance and permeability. In the rat model, within 20 min of intraperitoneal injection of endotoxin, blood and lung PAF concentrations increased for more than 120 min.[22] TNFα stimulates peritoneal macrophages, polymorphonuclear neutrophils, and vascular endothelial cells to synthesize and release PAF 1–4 h after TNFα administration *in vivo*.[23] The PAF and proteases remain as yet to be determined to clarify the mechanisms through which lidocaine posttreatment attenuated endotoxin-induced lung injury.

Cytokines, such as IL-1, TNF-α, and IL-6, might have been induced and released at the early phase of post endotoxin administration, namely before starting lidocaine treatment. These cytokines injure lung not directly but through activating many cell types, including neutrophils and macrophages. Lidocaine might be effective in the control of the next phase related to cytokines, because even pretreatment with lidocaine possessed partial effect on attenuation of cytokine secretion. Fletcher and Ramwell reported that posttreatment with the drug only for 3 h successfully decreased mortality (as assessed 72 h after endotoxin) due to endotoxin shock in baboons and dogs.[24] Our observations were inconsistent with their study. Lidocaine suppressed O_2^- production from neutrophils but did not cut off the cytokine cascade that may be responsible for the late phase of lung injury.

In conclusion, lidocaine administered after endotoxin slightly attenuated deterioration of oxygenation probably due to reduction of edema. A comparison of these findings with our previous findings[6] suggests that posttreatment with lidocaine may have only limited clinical application in patients with adult respiratory distress syndrome.

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